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BY

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THE INFLUENCE OF OXYGEN AND CARBON DIOXIDE ON THE GROWTH OF OPHIOBOLUS GRAMINIS IN PURE CULTURE[1]

By HURLEY FELLOWS

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INTRODUCTION

During the last few years Sewell and Melchers[2] have made some interesting observations on the destructiveness of take-all (*Ophiobolus graminis*) in the experimental wheat plots of the Kansas Agricultural Experiment Station at Manhattan, Kans. They noted that different kinds of cultivation in the plots apparently caused differences in the destructiveness of the take-all disease.

In inoculating wheat plants with *Ophiobolus graminis* the writer observed that sometimes, for no apparent reason, infection failed to develop. These inoculations were made and the inoculated plants kept in a greenhouse where the temperature was controlled and kept constantly favorable for infection. The soil moisture also was kept favorable for infection. Hence variation in the gas content of the soil suggested itself as a possible explanation of the apparent variations in the pathogenicity of the fungus.

Of the environmental factors that affect the development of a parasite in the soil, moisture, temperature, and acidity have received the most careful consideration from pathologists. Thus far little attention has been paid to the influence that changes in the gas content of the soil may exert on the growth and activity of the common soil-inhabiting parasites. As *Ophiobolus graminis* is influenced by different soil moistures and temperatures, which, in turn, affect the gas content of the soil, it was believed that, if the organism should prove sensitive to the variations in soil gases, its increase and activity might be controlled somewhat by any methods of cultivation that varied the gas content of the soil. With this in mind, the writer, in 1925, conducted a series of experiments in which pure cultures of *O. graminis* were grown in air containing different percentages of carbon dioxide and oxygen, respectively.

MATERIALS AND METHODS

The parasite was grown on solid media in Petri dishes and on liquid media in Erlenmeyer flasks. These were uniformly inoculated, each with a small piece of agar with new mycelium of the fungus, and placed in large glass jars closed with ground-glass tops. Through the top of

[1] Received for publication May 22, 1928; issued November, 1928. These investigations were conducted in cooperation with the Kansas Agricultural Experiment Station, Manhattan, Kans., and this paper is No. 274 of the Department of Botany and Plant Pathology.

[2] SEWELL, M. C., and MELCHERS, L. E. THE EFFECT OF ROTATION AND TILLAGE ON FOOTROT OF WHEAT IN KANSAS, 1920–1924. Jour. Amer. Soc. Agron. 16: 768–771, illus. 1924.

each jar three glass tubes were inserted and sealed in. One of these tubes extended to the bottom and served to admit the proper gas mixture that was to surround the cultures within. Another short tube permitted the removal of a small gas sample for analysis. The third tube had on its lower end a small rubber gas bag for keeping the barometric pressure inside the jar equal to that outside. The flask or Petri-dish cultures were placed in the bottom of the jar, which was then tightly sealed. A diagram of the apparatus is shown in Figure 1. The Petri-dish covers were raised slightly by means of small bent wires so placed that the gas in the Petri dishes could diffuse freely with that of the culture jar. The flasks were loosely plugged with cotton for the same reason.

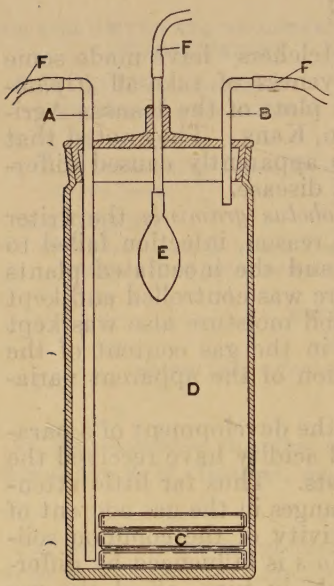

FIG. 1.—Diagram of a specimen jar used as a gas-control culture chamber. A, intake tube for gas mixtures; B, tube from which gas samples are taken for analysis; C, Petri dishes in which the organism is cultured; D, main portion of chamber in which various gas mixtures are retained; E, rubber gas bag used to keep the barometric pressure inside the jar equal to that on the outside; F, rubber-tube terminals upon which pinch cocks are placed

It was thought possible that the growth of the fungus might differ on different media, especially if one were solid and the other liquid, even though the concentrations of the gases of the surrounding atmosphere were constant. Accordingly, potato-dextrose agar and potato-dextrose decoction were selected as media. The growth measurements were made by weighing the dried mats of mycelium from the liquid medium and by measuring the diameters of the colonies on the agar medium.

Five different respective concentrations each of oxygen and carbon dioxide were used. (Tables 1 and 2.) When the concentration of carbon dioxide was varied that of oxygen was kept as near as possible to the percentage in the air, which by volume is approximately 21 per cent. However, when the oxygen was varied the concentration of carbon dioxide was 0.03 per cent or less. After the portions of oxygen or carbon dioxide necessary for a given percentage in a certain volume had been measured the remaining fraction of the volume was made up with nitrogen.

In the oxygen series, 22 to 24 agar cultures in Petri dishes were measured at each concentration, and 9 or 10 cultures from flasks were weighed. In the carbon-dioxide series 13 to 16 agar cultures in Petri dishes were measured at each concentration, and likewise for each concentration 3 or 4 flask cultures were weighed. It was not thought necessary to run more flask cultures in the carbon-dioxide series, as their responses were so similar to those of the agar cultures.

In these experiments where the oxygen or carbon dioxide was varied, the necessary gases were obtained from commercial pressure tanks. At the end of every 24-hour period the desired concentrations of oxygen and carbon dioxide, respectively, were made in a large graduated jar and then forced out of it into the culture jars by replacement with water. The measurements were easily made by replacing known quantities of water from the mixing jar with the gas

to be measured. The exact percentages of carbon dioxide and oxygen in the culture jars were measured with a portable gas-analysis apparatus. At the beginning of the experiments the measurements were made immediately before and immediately after a new gas supply was placed in the culture jar. It was soon found, however, that a 24-hour period of growth did not materially alter the percentage of oxygen or carbon dioxide; therefore the measurements were made only immediately after the new supplies of gases were placed in the jars. In all cases the cultures were grown at room temperature, about 21° C., for seven days.

TABLE 1.—*Growth of Ophiobolus graminis at different concentrations of oxygen*

[Age 7 days; kept at about 21° C.]

Oxygen concentration	Colonies on potato-dextrose agar		Oxygen concentration	Mycelial mats on potato-dextrose decoction		
	Number measured	Average diameter		Number weighed	Weight	
					Total	Average
Per cent		*Cm.*	*Per cent*		*Gm.*	*Gm.*
0.8	23	1.8	0.2	[a]	[a]	[a]
6.2	23	5.9	5.2	10	0.0912	0.0091
10.8	23	6.1	10.0	9	.3896	.0433
13.9	24	4.6	14.8	10	.6006	.0601
21.3	22	5.1	21.2	10	.6798	.0680

[a] Growth slight or none.

TABLE 2.—*Growth of Ophiobolus graminis at different concentrations of carbon dioxide*

[Age 7 days; kept at about 21° C.]

Carbon dioxide concentration	Colonies on potato-dextrose agar		Carbon-dioxide concentration	Mycelial mats on potato-dextrose decoction		
	Number measured	Average diameter		Number weighed	Weight	
					Total	Average
Per cent		*Cm.*	*Per cent*		*Gm.*	*Gm.*
0.25	14	9.04	0.90	3	0.3048	0.1016
3.59	13	8.62	3.17	4	.2630	.0658
5.19	14	4.61	5.57	3	.2696	.0899
11.80	6	6.69	11.50	4	.2276	.0569
16.75	14	5.50	18.02	4	.2120	.0530

RESULTS

GROWTH OF THE FUNGUS AT DIFFERENT CONCENTRATIONS OF OXYGEN

The results from growing cultures on potato-dextrose agar in different percentages of oxygen are given in Table 1 and are shown graphically in Figure 2. At all concentrations of oyxgen used except the lowest (0.8 per cent) the organism showed growth at the end of seven days. At 6.2 per cent and 10.8 per cent growth was slightly better than that in the controls at 21.3 per cent, whereas at 13.9 per cent the growth was slightly less than that in the controls.

In potato-dextrose decoction the fungus usually grew slightly, even at a very low oxygen concentration. The small block of agar used to inoculate the solution usually showed fuzziness even in a trace of oxygen (0.2 per cent). In general, there was a gradual diminution in growth with the decrease in oxygen. This behavior of the fungus contrasted strikingly with its action on the solid medium, where with certain exceptions, growth was approximately equal to

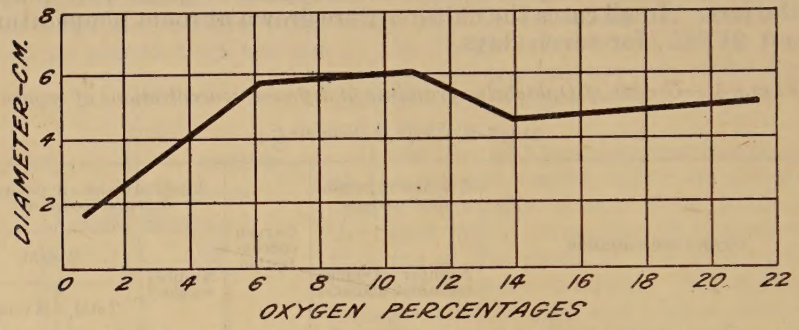

FIG. 2.—Growth of *Ophiobolus graminis* on potato-dextrose agar, at different percentages of oxygen. (Data in Table 1)

or better than the control until the oxygen was reduced below 6.2 per cent. (Table 1 and fig. 3.)

GROWTH OF THE FUNGUS AT DIFFERENT CONCENTRATIONS OF CARBON DIOXIDE

On potato-dextrose agar the fungus grew fairly well at all concentrations of carbon dioxide. In general, increased quantities of carbon dioxide usually diminished growth somewhat, but this diminution was not regular. When graphed the averages of growth measurements show a bimodal curve; that is, there was good growth

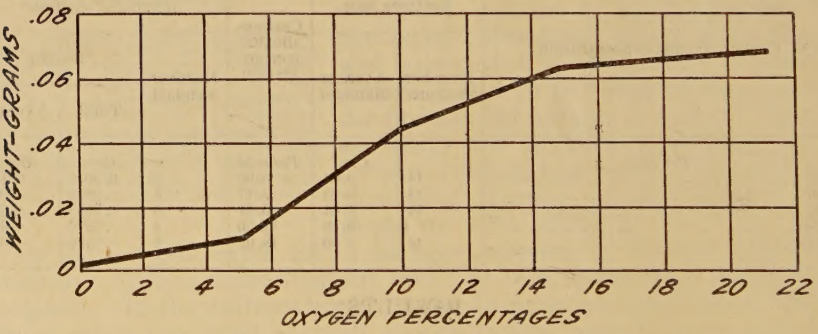

FIG. 3.—Growth of *Ophiobolus graminis* in potato-dextrose decoction, at different percentages of oxygen. (Data in Table 1)

at certain concentrations of carbon dioxide and poorer growth at an intervening concentration. The growth at 5.19 per cent was only about half as great as at 3.59 per cent. At 11.8 per cent it increased again about one-half more than at 5.19 per cent. High concentrations of carbon dioxide seemingly do not seriously impair the growth of *Ophiobolus graminis* when it is cultured on potato-dextrose agar. The growth at 16.75 per cent carbon dioxide was

about two-thirds of what it was at 0.25 per cent. (Table 2 and fig. 4.)

In general, the growth responses of the fungus in potato-dextrose decoction were similar to those on the solid medium. The curve of growth is bimodal and for the most part indicates a diminution of

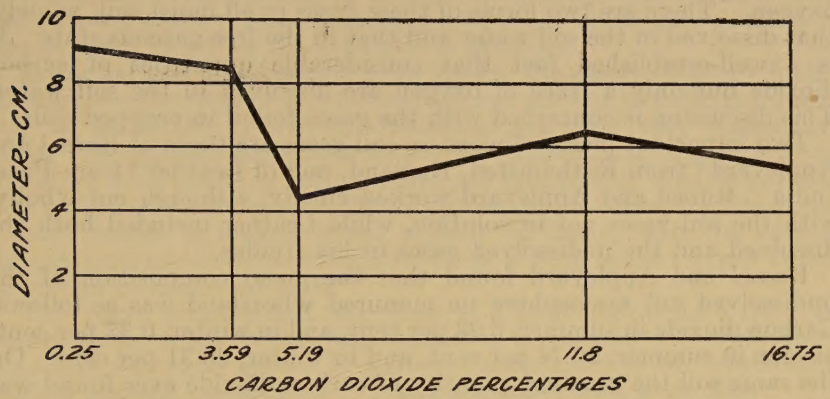

FIG. 4.—Growth of *Ophiobolus graminis* on potato-dextrose agar, at different percentages of carbon dioxide. (Data in Table 2)

growth with an increase in carbon dioxide. The two best points of growth are at 0.9 and 5.57 per cent. It may be noted that the second high point of growth has shifted toward the lower concentration as compared to that on the solid medium. The higher concentrations of carbon dioxide do not decrease the rate of growth very greatly,

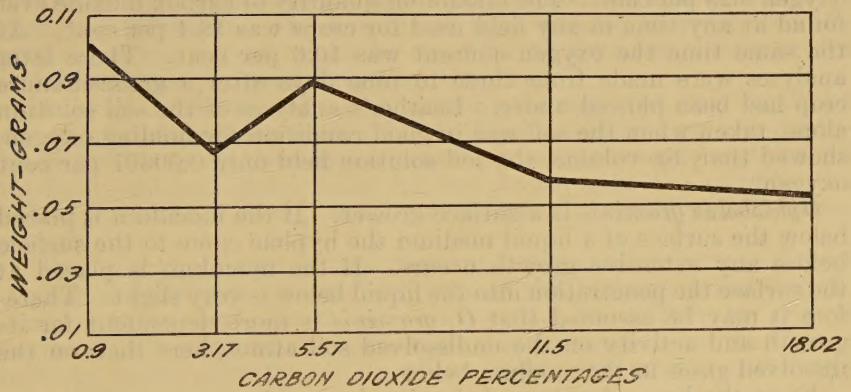

FIG. 5.—Growth of *Ophiobolus graminis* in potato-dextrose decoction, at different percentages of carbon dioxide. (Data in Table 2)

for, at the highest concentration used, growth was no less than half as great as that at the lowest concentration. One should not expect as much diminution at high percentages of carbon dioxide as at low percentages of oxygen, for oxygen is demanded in metabolism, while carbon dioxide is a by-product. (Table 2 and fig. 5.)

DISCUSSION

For a clearer understanding of the probable value of the experiments described in this paper it may be well to consider here the gaseous content of the soil. The two variable gases in the soil that mean most to the organisms that inhabit it are carbon dioxide and oxygen. There are two forms of these gases in all moist soil, namely, that dissolved in the soil water and that in the free gaseous state. It is a well-established fact that considerable quantities of carbon dioxide but only a trace of oxygen are dissolved in the soil water. This discussion is concerned with the gases found in cropped soils.

Two important pieces of work on soil gases are those of Russel and Appleyard [3] from Rothamsted, England, and of Leather [4] from Pusa, India. Russel and Appleyard worked chiefly, although not wholly, with the soil gases not in solution, while Leather included both the dissolved and the undissolved gases in his studies.

Russel and Appleyard found that the mean composition of the undissolved soil atmosphere on manured wheatland was as follows: Carbon dioxide in summer, 0.23 per cent, and in winter, 0.37 per cent; oxygen in summer, 20.74 per cent, and in winter, 20.31 per cent. On the same soil the greatest quantity of carbon dioxide ever found was 2.5 per cent in the early spring and the smallest quantity of oxygen about 18.4 per cent at the same time. In a water-logged soil the carbon dioxide was found to be as high as 9.1 per cent and the oxygen as low as 2.6 per cent. Such a condition, of course, is exceptional.

In the analyses reported by Leather both the dissolved and the undissolved gases were included. One of his representative samples was from a field growing corn (*Zea mays*). The maximum carbon dioxide content found at any time was 12.3 per cent and the minimum oxygen 6.28 per cent. The maximum quantity of carbon dioxide ever found at any time in any field used for crops was 18.4 per cent. At the same time the oxygen content was 10.6 per cent. These later analyses were made from three to nine days after a green-manure crop had been plowed under. Leather's analyses of the soil solution alone, taken when the soil was in good condition for holding oxygen, showed that, by volume, the soil solution held only 0.00607 per cent oxygen.

Ophiobolus graminis is a surface grower. If the inoculum is placed below the surface of a liquid medium the hyphae come to the surface before any extensive growth occurs. If the inoculum is placed at the surface the penetration into the liquid below is very slight. Therefore it may be assumed that *O. graminis* is more dependent for its growth and activity on the undissolved soil atmosphere than on the dissolved gases in the medium below.

From the laws of gaseous behavior, it would appear that the two soil atmospheres are not abruptly parted at the surface of water films, but that at this point there is more or less equilibrium between the two. This fact increases the difficulty of determining the percentages of the various gases to which the organism is exposed below the surface of its medium. Since *Ophiobolus graminis* is a surface grower, it is highly probable that the undissolved gases contribute

[3] RUSSEL, E. J., and APPLEYARD, A. THE ATMOSPHERE OF THE SOIL: ITS COMPOSITION AND THE CAUSES OF VARIATION. Jour. Agr. Sci. [England] 7: 1–48, illus. 1915.

[4] LEATHER, J. W. SOIL GASES. India Dept. Agr. Mem., Chem. Ser. 4: 85–134, illus. 1915.

most to its development. The small quantity of oxygen in the soil liquids, as shown by Leather's analyses, also indicates that the oxygen in the undissolved soil atmosphere is the more important. Laboratory technic has not yet been developed to the point where it is possible to determine the difference in the gas content of the surface and the deeper layers of thin aqueous films. Accordingly, one can not tell exactly what percentages of gases are in that portion of the medium in which the organism is growing.

As far as these experiments have shown, the gases ordinarily found in soils, whether free or in solution, are not present in sufficient quantity to affect the growth of *Ophiobolus graminis* very materially. Even the extreme quantities found by Russel and Appleyard on manured wheatlands in spring would not appreciably modify growth. The soil on which wheat is ordinarily grown in the wheat regions of the United States is seldom given organic fertilizers, and the carbon dioxide content therefore should rarely be as high or the oxygen as low as on the Rothamsted plots.

This paper presents but one phase of a pathological problem. To complete the study, wheat plants must be inoculated with *Ophiobolus graminis* and then grown in various concentrations of oxygen and carbon dioxide, respectively. Plans are under way to do this.

SUMMARY

Variations in the destructiveness of take-all in the field and irregularities in some greenhouse experiments suggested a series of tests to determine what effect different quantities of carbon dioxide and oxygen in the surrounding atmosphere might have on the growth of *Ophiobolus graminis*, the fungus causing take-all of wheat.

The cultures used were grown on potato-dextrose agar and potato-dextrose decoction. The two media were selected in order that the growth of the fungus might be observed on liquid and solid media when atmospheric conditions were the same.

On both the liquid and the solid media the organism grew in all the oxygen concentrations used. In the liquid medium, growth diminished gradually as the oxygen concentration decreased; on the solid medium, marked diminution did not occur until the oxygen was below 6 per cent. A very small percentage of oxygen greatly reduced growth.

The fungus grew well on both liquid and solid media when the carbon dioxide content was varied, although at the highest carbon-dioxide concentration used, 18.02 per cent, some diminution in growth occurred. In both of the carbon dioxide series; that is, the liquid-medium series and the solid-medium series, the growth curve was distinctly bimodal.

It is believed that the variations in carbon dioxide and oxygen as found in arable soils are not great enough to affect materially the growth of *Ophiobolus graminis*.

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